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Cloning and Expression Analysis of the Bovine Dentin Matrix Acidic Phosphoprotein Gene

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Abstract. The dentin matrix acidic phosphoprotein gene has been mapped to human chromosome 4q21 and mouse chromosome 5q21. Expression studies have implicated a role for this gene in the mineralization of dentin. In the current investigation, a cDNA encoding bovine dentin matrix acidic phosphoprotein has been cloned and sequenced. A comparison of the bovine gene with its rat counterpart has indicated that the genes are conserved (67.4% identity; 79.5% similarity), particularly in the region of presumed functional elements such as the hydrophobic signal peptide sequence, the cell attachment Arg-Gly-Asp tripeptide, and numerous serine residues which are likely candidates for phosphorylation. Zoo blot analysis further indicated that a similar gene is found in all mammalian species tested, but not in chicks. However, Northern analysis has indicated that in the cow the message is detectable at high levels in fetal bovine brain and cultured long bone as well as in odontoblasts. These results support a potential role for dentin matrix acidic phosphoprotein in dentinogenesis.

Key words: dentinogenesis, dentin, phosphoprotein, mineralization.

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Introduction

Acidic phosphorylated proteins have been shown to be prominent constituents of bone and dentin and have been implicated as playing a major role in the induction of mineralization in these tissues (Gorski, 1992). The acidic phosphoproteins of bone are rich in glutamic acid and aspartic acid residues with a lower serine content. Dentin contains a family of acidic phosphoproteins, termed the phosphophoryns, which are characterized by a very high content of aspartic acid and phosphorylated serine residues with a lower glutamic acid content (Sabsay *et al.*, 1991). Recently, a novel dentin matrix phosphoprotein (*dmp1*) has been cloned from a rat odontoblast cDNA library. Sequence analysis has shown that *dmp1* has an open reading frame of 1467 bp, which predicts a protein of 489 amino acid residues of which the 107 serines are the predominant constituent. The protein is also extremely acidic with 134 aspartic and glutamic acid residues. The composition of *dmp1* is therefore intermediate between that of the bone phosphoproteins and the phosphophoryns (George *et al.*, 1993).

Northern analysis has previously suggested that the *dmp1* message is essentially odontoblast-specific, although trace amounts of message were detectable in bone (George *et al.*, 1993). *In situ* hybridization studies have further suggested that *dmp1* mRNA expression is restricted to the fully differentiated odontoblasts which are engaged in active formation of the mineralized dentin (George *et al.*, 1994). The *dmp1* locus has been localized to mouse chromosome 5q21, which is syntenic with human chromosome 4q21, a region of the genome to which the autosomal dominant disorder of dentin formation, dentinogenesis imperfecta (DGI type II), has been mapped (Ball *et al.*, 1982; Crall *et al.*, 1992; Crosby *et al.*, 1995). Recently, a combination of genetic and physical mapping has enabled us to map the human DMP1 locus to chromosome 4q21 (Aplin *et al.*, 1995). Moreover, the isolation of a short tandem repeat polymorphism from a cosmid encompassing the DMP1 locus has permitted us to demonstrate that DMP1 is tightly linked to the DGI type II phenotype in two families, with no evidence of recombination between the two loci (Aplin *et al.*, 1995). To elucidate which regions of the *dmp1* gene are most highly conserved, we have isolated a cDNA clone encoding the bovine *dmp1* gene and compared the sequence with that of the rat (George *et al.*, 1993). We have further performed Northern analysis, to study the tissue distribution of bovine *dmp1* RNA, and zoo blot analysis to investigate the conservation of the gene during evolution.

Materials and methods

cDNA library screening

Bacteriophage from a bovine odontoblast cDNA library constructed in lambda ZAPII (Stratagene Cloning Systems, USA) were used to infect host strain *E. coli* XL-1 Blue and plated at 5 x 10^4 plaque-forming units/140 mm Petri dish. Primers designed from the rat cDNA sequence (George *et al.*, 1993) were used to amplify a 231 bp fragment from the 3′ end of the *dmp1* gene from rat genomic DNA with use of the polymerase chain reaction as detailed previously (Aplin *et al.*, 1995). The PCR product was radiolabeled by random priming (Feinberg and Vogelstein, 1983) and used to screen approximately 5 x 10^5 plaques. The membranes were washed by reduced stringency (1.0x SSC/0.1% SDS) at 65°C for 30 min. Autoradiography was performed at -70°C with double-intensifying screens for two days by means of Fuji RX film. Positive primary clones were purified by two additional rounds of screening and were subcloned into pBluescript.

Sequence analysis

Plasmids were restriction-mapped via a combination of single and double digests with *Bam*HI, *Eco*RI, *Hind*III, *Kpn*I, *Pst*I, *Sst*I, and *Xba*I. Suitable restriction fragments were subsequently subcloned into M13mp18/19 and sequenced by the dideoxy chain termination method (Sanger *et al.*, 1977) by means of the Sequenase version 2.0 kit (Amersham International, Cleveland, OH, USA).

Zoo blot analysis

Ten micrograms of genomic DNA from chick, mouse, dog, pig, sheep, cow, monkey, and human were digested with *Eco*RI. The DNA samples fractionated by agarose gel electrophoresis in tris/acetate buffer with 1.0% gels and transferred to Biodyne A membrane (Pall, UK) by standard methods (Sambrook *et al.*, 1989). The membranes were hybridized with radiolabeled DNA probes (Feinberg and Vogelstein, 1983) at 65°C. The membranes were washed to either 1.0 or 0.5x SSC at 65°C for 30 min. Autoradiography was performed at -70°C with double-intensifying screens for 1 to days with Fuji RX film.

Northern analysis

RNA was extracted, by standard methods, from fetal bovine material ranging in age from 3 to 5 months of gestation. Liver, skin, brain, and odontoblast RNA was extracted from intact tissue, while the bone preparation was obtained from cells cultured according to previously published methods (Robey and Termine, 1985). Ten µg of total RNA was separated by electrophoresis in formaldehyde denaturing agarose gels, blotted, and hybridized with radiolabeled *dmp1* or osteopontin cDNA as described previously (Ibaraki *et al.*, 1992). Unbound probe was removed when the membrane was washed in 2x SSC/0.1% SDS at 25°C followed by 0.1X SSC/0.1% SDS at 68°C for 30 min. Autoradiography was performed at -70°C with intensifying screens for 7 days with Kodak XAR film.

Results

Primers designed from the rat *dmp1* cDNA sequence (George *et al.*, 1993) were used to amplify a 231-bp fragment of the 3′ end of the gene from rat genomic DNA (Aplin *et al.*, 1995). When this PCR product was used to screen the bovine odontoblast cDNA library under reduced stringency, two positive clones, BO4 and BO11, were identified, which were found to be 2577 bp and 2305 bp, respectively. Restriction mapping of the clones revealed that they had the same restriction map. Sequence analysis confirmed that the clones were highly homologous to the rat *dmp1* gene; however, neither of the clones contained the entire coding sequence of the gene. A 1528-bp *Eco*R1/*Hind*III fragment of clone BO4 was therefore used to re-screen the odontoblast cDNA library, and three additional clones were identified, two of which, BO8 and BO12, were found to contain the entire coding sequence. Sequence analysis of the various clones revealed no evidence of alternative splicing. The complete nucleotide sequence of the coding region of the bovine *dmp1* gene is given in Fig. 1. A single open reading frame of 1530 bp predicts a highly acidic protein (isoelectric point = 3.95) of 510 amino acids. The molecular weight of the deduced protein is approximately 56 kDa. The 3′ untranslated region of 1379 bp, excluding the poly-A tail, contains a single polyadenylation signal (Fig. 1). Although the 3′ untranslated region is relatively AT-rich (59.4 %), it does not appear to contain any of the consensus sequences, such as UUAUUUA(U/A)(U/A), that have been shown to stimulate mRNA decay (Curtis *et al.*, 1995; Beelman and Parker, 1995). Ninety-two bp of 5′ untranslated region were also detected. A comparison of the deduced amino acid sequence of the cow and the rat *dmp1* genes indicates that the bovine gene is somewhat longer than its rat counterpart (510 residues compared with 489 residues); however, the genes are closely related, with 67.4% sequence identity and 79.5% similarity (Fig. 2). Several regions are conserved, including the hydrophobic signal peptide sequence, the cell attachment Arg-Gly-Asp tripeptide, and numerous serine residues which are likely candidates for phosphorylation by casein kinase I or II (Marshak and Carroll, 1991).

A 1794-bp *Sst*I/*Hind*III fragment from clone BO12, which contains the entire bovine *dmp1* sequence, was used to probe a zoo blot. The results of this analysis showed that this sequence was conserved in genomic DNA extracted from human, monkey, pig, sheep, and mouse (Fig. 3). Interestingly, no hybridization to chicken genomic DNA was detected (Fig. 3). The same fragment was used to probe a Northern blot containing total RNA isolated from a variety of tissues, and a transcript of approximately 3.0 kb was detected in brain, odontoblast, and bone (Fig. 4). At equal RNA loadings, the highest level of expression was detected in bovine brain, with lesser levels detected in RNA extracted from odontoblasts

GGATTTCCTCTTCAAGAACTTCAGCCTGA
TTGTTGAGCCTTTGGGGAAAAGTCTTTGTTAATTGAAGAGGGTAGGGGTGACACAGATGGCT

ATG AAG ACC ACC ATC CTG CTT ATG TTC CTG TGG GGA CTT TCC TGT GCT
Met Lys Thr Thr Ile Leu Leu Met Phe Leu Trp Gly Leu Ser Cys Ala

CTG CCA GTA GCC AGG TAT CAA AAT ACT GAA TCC AAG AGC TCT GAA GAA
Leu Pro Val Ala Arg Tyr Gln Asn Thr Glu Ser Lys Ser Ser Glu Glu

TGG AAG GGT CAT TTG GCT CAG ACA CCA ACA CCA CCT TTG GAG AGC AGT
Trp Lys Gly His Leu Ala Gln Thr Pro Thr Pro Pro Leu Glu Ser Ser

GAG TCA TCA GAA GAA AGT AAA CTT AGC TCA GAG GAA CAG GCA AAT GAA
Glu Ser Ser Glu Glu Ser Lys Leu Ser Ser Glu Glu Gln Ala Asn Glu

GAC CCC AGT GAC AGC ACA GAA TCC GAG GAG GTC CTG GGC CTT GAT GAT
Asp Pro Ser Asp Ser Thr Glu Ser Glu Glu Val Leu Gly Leu Asp Asp

CAG CAA CAT GTT CAT AGA CCA GCT GGC GGC CTC TCT CGG AGG GGA GGA
Gln Gln His Val His Arg Pro Ala Gly Gly Leu Ser Arg Arg Gly Gly

AGC GAA GGT GAT AAT AAA GAC GAT GAT GAA GAC GAG AGC GGA GAT GAC
Ser Glu Gly Asp Asn Lys Asp Asp Asp Glu Asp Glu Ser Gly Asp Asp

ACC TTT GGG GAT GAT GAT GGT GGC CCA GGA CCC GAA GAG AGA CGA TCA
Thr Phe Gly Asp Asp Asp Gly Gly Pro Gly Pro Glu Glu Arg Arg Ser

GGA GGG GAC TCC AGG CTT GGA AGC GAC GAA GAC TCG GCT GAC ACC ACA
Gly Gly Asp Ser Arg Leu Gly Ser Asp Glu Asp Ser Ala Asp Thr Thr

CGA TCC AGG GAA GAC AGC ACC CCA CAA GGG GAT GAG GGG GCC CGT GAT
Arg Ser Arg Glu Asp Ser Thr Pro Gln Gly Asp Glu Gly Ala Arg Asp

ACC ACC AGC GAG AGC AGG GAC CTT GAC CGT GAG GAT GAG GGG AAC AGC
Thr Thr Ser Glu Ser Arg Asp Leu Asp Arg Glu Asp Glu Gly Asn Ser

AGG CCC GAG GGC GGT GAC TCC ACT CCA GAC AGC GAC AGT GAG GAG CAC
Arg Pro Glu Gly Gly Asp Ser Thr Pro Asp Ser Asp Ser Glu Glu His

TGG GTG GGA GGC GGC AGT GAG GGG GAC AGC AGC CAC GGG GAT GGC TCT
Trp Val Gly Gly Gly Ser Glu Gly Asp Ser Ser His Gly Asp Gly Ser

GAG TTC GAC GAT GAA GGG ATG CAG AGC GAT GAC CCG GGC GCC TAC AGG
Glu Phe Asp Asp Glu Gly Met Gln Ser Asp Asp Pro Gly Ala Tyr Arg

AGC GAG AGG GGC AAC TCC CGA ATA AGC GAT GCC GGC CTC AAG TCA ACA
Ser Glu Arg Gly Asn Ser Arg Ile Ser Asp Ala Gly Leu Lys Ser Thr

CAA TCG AAA GGG GAC GAT GAG GAG CAG GCA AGC ACC CAG GAT TCC CAT
Gln Ser Lys Gly Asp Asp Glu Glu Gln Ala Ser Thr Gln Asp Ser His

GAG AGC CCA GCA GCC GCG TAT CCC CGC AGG AAA TTC TTC CGG AAG TCT
Glu Ser Pro Ala Ala Ala Tyr Pro Arg Arg Lys Phe Phe Arg Lys Ser

CGT CTT CCT GAG GAA GAT GGC AGA GGG GAG CTT GAC GAT AGC CGC ACG
Arg Leu Pro Glu Glu Asp Gly Arg Gly Glu Leu Asp Asp Ser Arg Ser

Figure 1 (Part 1). Nucleotide sequence of the bovine dentin matrix acidic phosphoprotein cDNA and the deduced amino acid sequence of the protein. The potential signal peptide region is underlined, the RGD sequence is indicated in boldface, and the polyadenylation consensus sequence is italicized.

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ATA GAA GTC ATG AGT GAC TCC ACC GAA AAC CCC GAC TCC AAA GAA GCC
Ile Glu Val Met Ser Asp Ser Thr Glu Asn Pro Asp Ser Lys Glu Ala

GGC CTT GGC CAA TCC AGG GAA CAC AGC AAG AGT GAA TCT CGA CAA GAG
Gly Leu Gly Gln Ser Arg Glu His Ser Lys Ser Glu Ser Arg Gln Glu

AGT GAG GAG AAC CGG TCC CCG GAA GAC AGT CAG GAT GTC CAA GAC CCC
Ser Glu Glu Asn Arg Ser Pro Glu Asp Ser Gln Asp Val Gln Asp Pro

AGC AGC GAG TCT AGT CAA GAG GTC GAC CTG CCT TCT CAA GAA AAC AGT
Ser Ser Glu Ser Ser Gln Glu Val Asp Leu Pro Ser Gln Glu Asn Ser

AGC GAA TCT CAG GAA GAG GCG CTC CAT GAG TCC AGG GGT GAC AAC CCC
Ser Glu Ser Gln Glu Glu Ala Leu His Glu Ser Arg Gly Asp Asn Pro

GAC AAC GCC ACC AGT CAC TCC AGA GAA CAT CAG GCG GAT AGT GAG TCC
Asp Asn Ala Asn Ser His Ser Arg Glu His Gln Ala Asp Ser Glu Ser

AGT GAG GAG GAC GTG TTG GAT AAG CCC TCC GAT TCA GAG AGC ACA TCC
Ser Glu Glu Asp Val Leu Asp Lys Pro Ser Asp Ser Glu Ser Thr Ser

ACA GAG GAA CAG GCT GAC AGC GAA TCC CAT GAG AGC CTC AGG TCC TCG
Thr Glu Glu Gln Ala Asp Ser Glu Ser His Glu Ser Leu Arg Ser Ser

GAG GAG AGC CCA GAG TCC ACT GAA GAG CAG AAC AGT TCT AGC CAG GAG
Glu Glu Ser Pro Glu Ser Thr Glu Glu Gln Asn Ser Ser Ser Gln Glu

GGC GCC CAG ACC CAG AGC CGG AGC CAG GAG AGC CCG TCT GAG GAG GAC
Gly Ala Gln Thr Gln Ser Arg Ser Gln Glu Ser Pro Ser Glu Glu Asp

GAT GGT AGC GAT TCC CAA GAC AGC AGC AGA TCG AAA GAG GAC AGC AAC
Asp Gly Ser Asp Ser Gln Asp Ser Ser Arg Ser Lys Glu Asp Ser Asn

TCG ACC GAG AGC GTG TCA AGC AGT GAG GAA GAG GCC CAA ACT AAA AAC
Ser Thr Glu Ser Val Ser Ser Ser Glu Glu Glu Ala Gln Thr Lys Asn

ACT GAA GTA GAA AGC AGA AAA TTA ACA GTC GAT GCG TAC CAC AAC AAA
Thr Glu Val Glu Ser Arg Lys Leu Thr Val Asp Ala Tyr His Asn Lys

CCC ATC GGA GAT CAG GAT GAC AAT GAT TGC CAA GAT GGC TAT TAG
Pro Ile Gly Asp Gln Asp Asp Asn Asp Cys Gln Asp Gly Tyr  *

CATGGGCGTGCCTGAGCGCCTCTCACAGACAGGCGTCCTGGAGGCTGGAGACTAGGGAAAATC
ATAACCGTAATTTATTGACGTTTGTATCAGAAGAATAGCCTGAGGCCATTTCATTCTGAAAGG
AAATGCTCGATGTTATACTTGTTTGTGTCTAGGGTGTCATCAAACCATAGAGGTTTCAATAAT
GGAAAATGTCACTAGAACACCCTCCATGGGAGACCTAAGCAAGGAAAGATGTGTGTTGTTGCT
TCACAGCTGAAATAGTTCCTAACTCATTAACCTAACGCACAGTTACACAGCTTCTGCTATGTA
CCAGGCACTGCACTGGGTGGCGAGGAGGTCAAGAAGCTTAAAGACTTGTCTCTTGCTCCTGAG
GAGAAGGTTTTGTTGTTGTTCAGGCTCTAAGTTGTGTCCATCTCTTTGTGACCCATGGACTGC
AGCACACCAGGCTCCTCTGTCCTCTCCTAACTCCGGAGTTTGCTCAAATTCATGTCCATTGAG
TCAGTGATGCTATCTAACCATCTCATCCTCTGTTGCCCCCTTCTCCTTGTGCCTTCAATCTTT
CCCAACATCAGGGTCTTTTCCAATGAGTGGGCTCTCCGCATCAGGTGGCCAAAGTATTGGAGT
TTCAGCTTCAGCATCAGTCCTTCCAATGAATATTCAGGGTTAATTTCCTTCAGAATTGACTAG
TTTGATCTCTGAGAAGCTTATAGAGGGTAAATAAAGCTGAAAAGGGATAAATATGACAAAAGC
AAGAATTATTACAATCCAGTGTGGTGATTGCTATTAACACAGATAATGAACAAAACATATGGA
TTCACAGAAGAGGGCGCACTTTGCCTTGAGAATTAGAGAGGGCTTCACAGCAGGAAAGACACT
GGAGTCAGAACGTTGCATGGATTTCAACATTTAAGGCTAGACAATTCTATTTATCCTTGGTA
TCAGCAATAGAAAATCCTATATGAATAAACATTTAGTTTGTGAAAGTGTTTTCAAAATAGGGC
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Figure 1 (Part 2). Nucleotide sequence of the bovine dentin matrix acidic phosphoprotein cDNA and the deduced amino acid sequence of the protein. The potential signal peptide region is underlined, the RGD sequence is indicated in boldface, and the polyadenylation consensus sequence is italicized.

GCAGTAATGTACTTCAGATAAGCAAAAATTCTTTTTTCAATTAACCCTTTTTGATTTTTTCCT
TTTTTGTGTGTGTATGTGTGTGTGTGACACTCCCATGAGCGTCACCTCCCTTACCACTGGTGA
TGATATGGAGGACTGGGTGGGGGAGGGTGGAAAAATGAAATTCCTCTTTAAATAGACAACTAT
CAGCTTAATTTTTTTCTAAGATCAATCCATGTGCAAAGCTAGAAAAACTGTTCTTGGAGTACT
CTATTTGTGTTGCACATAGCAAAATTGTGTGTATAGAATTCAATTGATTTTTGCCTAATACCC
TTTGAAATGTTACCTCAACCTAAAATATTTGTTTTGT*AATAAA*GATTATAATATCCAAAAAAA
AAAAAAAAAAAA

Figure 1 (Part 3). Nucleotide sequence of the bovine dentin matrix acidic phosphoprotein cDNA and the deduced amino acid sequence of the protein. The potential signal peptide region is underlined, the RGD sequence is indicated in boldface, and the polyadenylation consensus sequence is italicized.

and cultured long bone (Fig. 4). While no data are available on the expression of bovine *dmp1* in alveolar bone, no expression was detected in RNA extracted from bovine liver, skin, or ameloblasts (data not shown). As a positive control in the experiment, the blot was probed with osteopontin, and this showed that a 2.0-kb transcript was detectable in RNA extracted from skin, brain, and cultured bone (Fig. 4).

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M K T T I L L M F L W G L S C A L P V A R Y Q N T E S K S S E E W K
* * * V * * * T * * * * * * * * * * * * * * * * * * * E * * * * R T

G H L A Q T P T P P L E S S E S S E E S K L S S E E Q A N E D P S D
* N * * * S * P * * M - - - - - - - - - - - - - - - - * * S * H T *

S T E S E E V L G L D D Q Q H V H R P A G G L S R R G G S E G D N K
* S * * G * E * * S * R S * - - Y * * * * * * * K S A G M D A * - *

D D D E D E S G D D T F G D D D G G P G P E E R R S G G D S R L G S
E E * * * D * * * * * * * * E * N * * * * * * * Q W * * P * R L D *

D E D S A D T T R S R E D S T P Q G D E G A R D T T S E S R D L D R
* * * * * * * * Q * S * * * * S * - E N S * Q * * P * D * K * - H H

E D E G N S R P E G G D S T P D S D S E E H W V G G G S E G D S S H
S * * A D * * * * A * * * * Q * * E * * * Y R * * * * * * * E * * *

G D G S E F D D E G M Q S D D P G A Y R S E R G N S R I S D A G L K
* * * * * * * * * * * * * * * * * S T * * D * * H T * M * S * D I S

S T Q S K G D D E E Q A S T Q D S H E S P A A A Y P R R K F F R K S
* E E * * * - * H * P T * * * * * D D * Q D V E F S S * * S * * R *

R L P E E D G R G E L D D S R S I E V M S D S T E N P D S K E A G L
* V S * * * D * * * * A * * N * R * T Q * V * * * D F R * * - - - -

G Q S R E H S K S E S R Q E S E E N R S P E D S Q D V Q D P S S E S
- - - - * E * R * * T Q E D T A * T Q * Q * * * P E G * * * * * * *

S Q E V D L P S Q E N S S E S Q E E A L H E S R G D N P D N A N S H
* E * A G E * * * * S * * * * * * G V A S * * * * * * * * * T S Q T

S R E H Q A D S E S S E E D V L D K P S D S E S T S T E E Q A D S E
G - - D * R * * * * * * * * R * N T F * S * * * Q * * * * * G * * *

S H E S L R S S E E S P E S T E E Q N S S S Q E G A Q T Q - - - - -
* N * * * S L * * * * Q * * A Q D E D * * * * * * L * S * S A S R E

S R S Q E S P S E E D - - - - - - G S D S Q D S S R S K E D S N S T
* * * * * * Q * * * S R S E E N R D * * * * * * * * * * * E * * * *

E S V S S S E E E A Q T K N T E V E S R K L T V D A Y H N K P I G D
G * T * * * * * D N H P * * I * A D N * * * I * * * * * * * * * * *

Q D D N D C Q D G Y
* * * * * * * * * *
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Figure 2. Comparison of the deduced amino acid sequences of the cow and the rat *dmp1* genes. The cow sequence is written above that of the rat, which is taken from George *et al.* (1993). Stars (*) indicate amino acid identity, letters indicate differing amino acids, and dashes indicate absent residues.

Discussion

In the current investigation, the bovine *dmp1* gene has been cloned from an odontoblast cDNA library. Previously, Northern analysis in the rat has suggested that the gene is essentially odontoblast-specific (George *et al.*, 1993). *In situ* hybridization studies have further shown that *dmp1* mRNA expression is restricted to the fully differentiated odontoblasts which are engaged in active formation of the mineralized dentin, although this was not an exhaustive developmental study (George *et al.*, 1994). In the current investigation, Northern analysis has shown that *dmp1* message is also detectable in bovine odontoblasts. Nevertheless, whereas George *et al.* (1993) did not detect *dmp1* message in RNA extracted from rat brain, we have shown that bovine *dmp1* is expressed at a high level in this tissue. Moreover, we have also detected *dmp1* expression in RNA extracted from cultured bone; conversely, George *et al.* (1993) detected only trace amounts of *dmp1* message in tibia, and none at all in calvarial RNA. While the RNA used in the current study was isolated from cultured long bones (Robey and Termine, 1985), the use of this system for the study of osteogenesis has been validated previously (Ibaraki *et al.*, 1992). More specifically, the cultured bone cells used in this study have previously been shown to retain many traits typical of mature osteoblasts, including (1) cAMP response to parathyroid hormone and (2) extensive elaboration of matrix, including the production of the non-collagenous acidic RGD-containing bone sialoprotein. Since the late expression of BSP protein is generally considered to be a "late marker" of bone tissue differentiation (Gehron Robey *et al.*, 1992), it is interesting to speculate that *dmp1* follows a similar pattern of expression in mineralized tissue and could also represent a

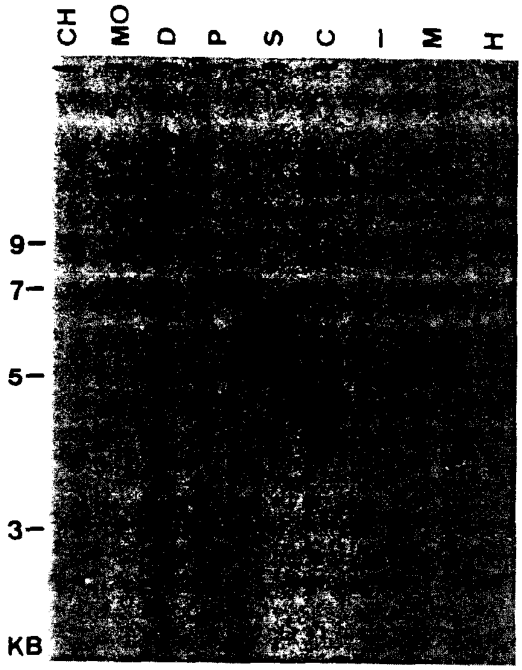

Figure 3. Conservation of the dentin matrix acidic phosphoprotein gene across species as indicated by zoo blot analysis. The blot was probed with an 1794-bp *SstI/HindIII* fragment of cDNA clone BO12, which contains the entire coding sequence of the bovine *dmp1* gene, washed to 0.5X SSC/0.1% SDS at 65°C and exposed for 2 days. CH = chicken; MO = mouse; D = dog; P = pig; S = sheep; C = cow; - = no DNA; M = monkey; and H = human.

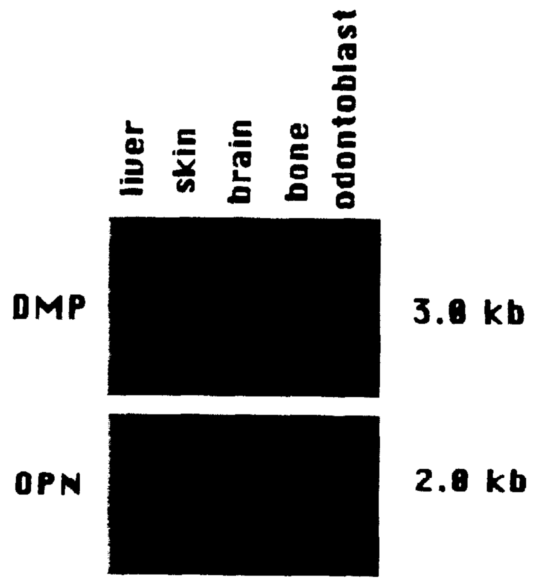

Figure 4. Northern analysis of bovine dentin matrix acidic phosphoprotein (DMP) and osteopontin (OPN) in a variety of tissues. The approximate transcript size is indicated in the right side of the figure.

"late" mineralized tissue marker. The brain material used for this study was obtained from a fetal cow that was approximately the same age as the donor for the bovine bone material (3 to 5 months' gestation). It is possible, therefore, that *dmp1* expression seen in this fetal material resulted from a transient expression of the gene and, in turn, might disappear or "down regulate" as the animal matures. A similar phenomenon occurs with the expression of osteonectin, another highly abundant, acidic, non-collagenous protein in mineralized tissue. Multiple tissues and organs express this protein in the embryonic stage, while in the adult, the expression pattern is considerably more restricted in tissue location (Young *et al.*, 1993). To obtain a more complete picture of the temporal and spatial patterns of *dmp1* expression, and how it relates to other matrix genes, investigators should perform *in situ* hybridization on sections of developing hard and soft tissues. Such experiments are currently ongoing with mouse tissues. In preliminary studies, a broader pattern of expression was seen compared with that previously reported by George *et al.* (1994). Finally, it is not clear at this time whether the mRNA for *dmp1* is translated into protein in either brain or bone. To answer this question, experiments are under way to make a recombinant fusion protein for subsequent antisera production.

Zoo blot analysis indicated that a similar gene is present in the genomes of a variety of mammals, but is absent from the chicken genome. The fact that chickens do not develop teeth provides further evidence for a role for this gene in odontogenesis; however, as stated earlier, it would be important to document precisely the expression patterns of this gene, and its protein product, during mammalian craniofacial/dental development.

Given that both the DGI type II and DMP1 loci have been mapped to human chromosome 4q21 and are tightly linked (Aplin *et al.*, 1995), the DMP1 locus would appear to be a strong candidate for the gene mutated in DGI type II. In this regard, the isolation of a bovine *dmp1* cDNA will prove to be useful in the isolation of its human homologue. Moreover, comparison of the bovine sequence with that of the rat indicates that the two genes are closely related, with the hydrophobic signal peptide sequence, the cell attachment Arg-Gly-Asp tripeptide, and numerous serine residues which are likely candidates for phosphorylation by casein kinase I or II all conserved. All of these regions would therefore appear to be potential sites for mutations in DGI type II. Future studies will concentrate on the isolation of the human DMP1 gene and an assessment of its potential role in the pathogenesis of DGI type II.

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